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Penicillinase-producing Neisseria gonorrhoeae isolates from different localities in south east Asia

Susceptibility to 15 antibiotics

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SUMMARY Sixty four penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and 24 non-penicillinase-producing (non-PPNG) strains isolated from six different south east Asian localities were tested by the agar dilution method against 15 antibiotics. All isolates were susceptible to spectinomycin and sulphamethoxazole-trimethoprim (19 : 1 ratio). A large proportion of both PPNG and non-PPNG strains showed, however, a decreased susceptibility to tetracycline, kanamycin, and erythromycin: 49% with minimum inhibitory concentrations (MICs) of tetracycline $\geqslant 2$ μg/ml, 34% with MICs of kanamycin $\geqslant 32$ μg/ml, and 80% with MICs of erythromycin $\geqslant 2$ μg/ml. These MIC cut-off values were chosen since they are close to the highest concentrations of these antibiotics attainable in serum after drug administration. Resistance to these antibiotics was not related to penicillinase production and does not appear to be confined to gonococci isolated from one particular locality. Strains showing resistance concurrently to two or three of these drugs were often isolated from different south east Asian countries. All eight cephalosporins tested were effective against both PPNG and non-PPNG strains. On a weight to weight basis the new cephalosporins—namely, moxalactam, cefoperazone, cefotaxime, and ceftriaxone—were the most effective. In contrast to those of cefoxitin, cefuroxime, moxalactam, and cefoperazone the MICs of cefamandole, cefotaxime, and ceftriaxone were significantly affected when the inoculum size was increased from 10^3 to 10^6 colony forming units (cfu).

Introduction

Since the emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) isolates in 1976,[1 2] there has been a continuously high prevalence of these strains in most south east Asian countries.[3-5] The high incidence of strains totally resistant to penicillin treatment causes worldwide concern since epidemiological and plasmid analyses have shown that PPNG infections in North America[6] and the Netherlands[7] were often traceable to sources from the Far East. More recently, and for the first time, PPNG strains bearing the Asian-type plasmid were found to have spread within a local community in England.[8] While the reasons for the high incidence of PPNG in the Far East remain uncertain, it has long been observed that gonococci isolated from south east Asian countries were generally more resistant to a number of antibiotics than those isolated in Western countries.[9 10] This emphasises the need for continuous surveillance of the antibiotic sensitivities of these strains. We report the in vitro activities of 15 antimicrobial agents against recent isolates of 64 PPNG and 24 non-PPNG strains obtained from different south east Asian countries.

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Materials and methods

BACTERIAL STRAINS

N gonorrhoeae strains were randomly selected pretreatment isolates obtained from various hospitals

and clinics in six south east Asian localities: 20 PPNG strains were obtained from Hong Kong, 12 from Indonesia, 16 from Malaysia, nine from Thailand, five from Singapore, and two from the Philippines; 10 non-PPNG strains were obtained from Indonesia, two from Malaysia, 11 from Thailand, and one from Singapore. Except for one PPNG and two non-PPNG strains from Thailand which were collected in 1978 all gonococcal strains were isolated during the past two years. All PPNG and non-PPNG strains were lyophilised and sent to Hong Kong until ready for susceptibility testing. Cultures were confirmed to be *N gonorrhoeae* by colonial morphology, Gram stain, oxidase reaction, and carbohydrate utilisation tests. β-lactamase activity of PPNG strains was detected by the chromogenic cephalosporin test.[11]

ANTIBIOTIC SUSCEPTIBILITY

Susceptibility of *N gonorrhoeae* strains to benzylpenicillin, cefuroxime, cephalothin (Glaxo), tetracycline, ampicillin, kanamycin (Sigma), moxalactam, cefamandole (Eli Lilly), sulphamethoxazole-trimethoprim, ceftriaxone (Hoffmann-La Roche), erythromycin (Abbott), spectinomycin (Upjohn), cefoxitin (Merck Sharp & Dohme), cefotaxime (Hoechst-Roussel), and cefoperazone (Pfizer) were tested by the agar dilution method. Doubling dilutions of each antibiotic were incorporated in GC medium base supplemented with 1% haemoglobin and 1% GC supplement without antibiotic (Oxoid). This medium was used for all antibiotics except sulphamethoxazole-trimethoprim (19:1 ratio) which was tested on diagnostic sensitivity test agar (Oxoid) containing 5% lysed horse blood and 1% defined supplement (Oxoid).

Inocula were prepared from an overnight growth on GC medium. Several colonies of each test and control strain (*N gonorrhoeae* 76-073389, 77-083718 from the Centers for Disease Control, Atlanta, USA, and *Staphylococcus aureus* NCTC 6571) were suspended in tryptic soy broth until the turbidity matched that of a 0·5 McFarland standard. The suspension was further diluted to give a final inoculum of 10^3 cfu per spot and inoculated on to the test or control plates (without antibiotic) with a Dynatech multipoint inoculator. When the effect of inoculum size was tested, an inoculum of 10^6 cfu was applied to each spot. After the inocula had dried, the plates were incubated in a 5% CO_2 incubator for 24 hours and the MICs taken as the highest dilution of antibiotics which gave no growth or only a single colony.

Results

The MIC distribution of seven antibiotics against 64 PPNG and 24 non-PPNG strains isolated from six different south east Asian localities is shown in table I. For penicillin, 79% of the non-PPNG strains showed slightly decreased susceptibility (MICs ≥0·125 μg/ml), but none of the isolates required concentrations >1 μg/ml for inhibition. In contrast, the 64 PPNG strains tested had MICs of penicillin ranging from 1 to 32 μg/ml. Strains showing resistance to penicillin also had higher MICs of ampicillin for inhibition of growth. With the exception of penicillin and ampicillin which clearly

TABLE I *Distribution of minimum inhibitory concentrations (MICs) of seven antimicrobial agents against penicillinase-producing and non-penicillinase-producing N gonorrhoeae strains isolated from six south east Asian localities*

Antibiotics	Penicillinase production*	No of strains with MIC (μg/ml) of:											
		0·03	0·06	0·125	0·25	0·5	1	2	4	8	16	32	64
Penicillin	+						6	8	16	15	13	6	
	−	2	3	2	8	7	2						
Ampicillin	+							4	1	10	23	23	3
	−			8	6	7	3						
Tetracycline	+					6	22	25	10	1			
	−					6	11	4	3				
Erythromycin	+		1	1	1		9	9	19	20	4		
	−					3	3	2	8	6	2		
Kanamycin	+					2		1	3	14	22	17	5
	−							1	2	6	7	5	3
Spectinomycin	+						1	7	6	23	22	5	
	−							1	3	14	6		
Sulphamethoxazole-trimethoprim†	+						3	17	24	11	7	2	
	−							4	8	10	2		

\+ Positive; − negative

*64 PPNG strains were obtained from Hong Kong (20), Indonesia (12), Malaysia (16), Thailand (9), Singapore (5), and the Philippines (2); 24 non-PPNG strains were obtained from Indonesia (10), Malaysia (2), Thailand (11), and Singapore (1).

†19:1 ratio.

separated PPNG from non-PPNG strains, the MICs of the other antibiotics tested showed a more or less parallel distribution between these two types of strains. Spectinomycin and sulphamethoxazole-trimethoprim (19 : 1 ratio) were effective against both PPNG and non-PPNG strains and all isolates were inhibited at a concentration of 32 μg/ml.

A large proportion of both PPNG and non-PPNG strains showed decreased susceptibility to tetracycline, kanamycin, and erythromycin: 56% and 29% of the strains respectively had MICs of tetracycline ⩾2 μg/ml; 34% and 33% had MICs of kanamycin >32 μg/ml; while 81% and 75% had MICs of erythromycin ⩾2 μg/ml (table I).

High resistance to tetracycline, erythromycin, and kanamycin did not appear to be confined to gonococci isolated from a particular locality (table II). Although the proportion of PPNG strains isolated from Hong Kong showing resistance to kanamycin appeared to be smaller than that of those obtained from other countries, the difference was not statistically significant ($\chi^2 = 7{\cdot}6597$; $p<0{\cdot}1>0{\cdot}05$).

Table III shows the number of strains from different localities showing resistance to either tetracycline, erythromycin, or kanamycin alone or a combination of two or three of these antibiotics. It is apparent that concurrent resistance to these antibiotics is common. Of a total of 64 PPNG and 24 non-PPNG strains, 58% and 46% respectively were concurrently resistant to two or three of these drugs.

The MIC distribution of eight cephalosporins against the PPNG and non-PPNG strains is shown in table IV. All the compounds tested were effective against both types of strains and none of the isolates had a MIC >4 μg/ml. On a weight to weight basis, cefoperazone, moxalactam, cefotaxime, and ceftriaxone were the most effective and all strains were inhibited at an antibiotic concentration of 0·25 μg/ml. Of both the older generation cephalosporins, cephalothin showed an in vitro activity against both PPNG and non-PPNG strains comparable with that of cefamandole. In contrast to cefoxitin, cefuroxime, moxalactam, and cefoperazone, when the inoculum size was increased from 10^3 to 10^6 cfu (table V) a greater than fourfold increase in MICs was observed with cefamandole, cefotaxime, and ceftriaxone. For cefamandole the effect of inoculum size was more apparent for PPNG strains.

Discussion

Reyn[9] and Willcox[10] were probably the first to note that *N gonorrhoeae* strains isolated from different south east Asian countries were more resistant to a number of commonly used antibiotics compared with gonococci isolated from Western countries. More recently, Thornsberry *et al*[12] have also reported that both PPNG and non-PPNG strains obtained from

TABLE II *Resistance to tetracycline, erythromycin, and kanamycin of penicillinase-producing strains of N gonorrhoeae: comparison of strains isolated from different south east Asian localities*

Locality	No of strains	No (%) of strains resistant to: Tetracycline (MICs ⩾2 μg/ml)	Erythromycin (MICs ⩾2 μg/ml)	Kanamycin (MICs ⩾32 μg/ml)
Hong Kong	20	11 (55)	14 (70)	2 (10)
Indonesia	12	5 (42)	8 (67)	5 (42)
Malaysia	16	9 (56)	15 (94)	6 (38)
Others*	16	11 (69)	15 (94)	9 (56)
Total	64	36 (56)	52 (81)	22 (34)

*This included nine PPNG strains from Thailand, five from Singapore, and two from the Philippines.

TABLE III *Resistance to tetracycline (T), kanamycin (K), and erythromycin (E), either alone or in combination, of penicillinase-producing and non-penicillinase-producing N gonorrhoeae strains*

Source of PPNG strains	No tested	No of strains showing resistance to: TKE	TE	KE	T	K	E	No of fully sensitive strains
Hong Kong	20	1	7	0	3	1	6	2
Malaysia	16	5	4	1	0	0	5	1
Indonesia	12	3	2	1	0	1	2	3
Other areas*	16	6	5	2	0	1	2	0
Non-PPNG strains†	24	2	5	4	0	2	7	4

*This included nine PPNG strains from Thailand, five from Singapore, and two from the Philippines.
†This included 10 non-PPNG strains from Indonesia, two from Malaysia, 11 from Thailand, and one from Singapore.

TABLE IV *Distribution of minimum inhibitory concentrations (MICs) of eight cephalosporins against penicillinase-producing and non-penicillinase-producing N gonorrhoeae strains isolataed from six south east Asian localities*

Antibiotics	Penicillinase production*	No of strains with MIC (μg/ml) of: 0·0018	0·0037	0·0075	0·015	0·03	0·06	0·125	0·25	0·5	1	2	4
Cephalothin	+						1		5	13	27	14	4
	−								2	6	7	8	1
Cefamandole	+							1	10	29	9	12	3
	−							3	6	7		7	1
Cefoxitin	+							7	3	45	9		
	−							3	1	17	3		
Cefuroxime	+				15	7	36	6					
	−				4	1	11	5	2		1		
Cefoperazone	+	1	1	3	6	20	20	11	2				
	−		2	1		3	9	9					
Moxalactam	+				5	40	16	2	1				
	−				2	11	10	1					
Cefotaxime	+	14	15	21	8	5	1						
	−	3	3	2	8	7	1						
Ceftriaxone	+	32	26	5	1								
	−	6	6	11	1								

*64 PPNG strains were obtained from Hong Kong (20), Indonesia (12), Malaysia (16), Thailand (9), Singapore (5), and the Philippines (2); 24 non-PPNG strains were obtained from Indonesia (10), Malaysia (2), Thailand (11), and Singapore (1).

TABLE V *Effects of increase in inoculum size on the range of minimum inhibitory concentrations (MICs) and MIC_{90} of seven cephalosporins against 55 penicillinase-producing (PPNG) and 24 non-penicillinase-producing (non-PPNG) strains of N gonorrhoeae*

	PPNG				Non-PPNG			
	10^3 colony forming units		10^6 colony forming units		10^3 colony forming units		10^6 colony forming units	
Antibiotics	MIC range	MIC_{90}	MIC range	MIC_{90}	MIC range	MIC_{90}	MIC range	MIC_{90}
Cefoxitin	0·125 – 1	0·61	0·125 – 1	0·59	0·125 – 1	0·58	0·125 – 1	0·85
Cefuroxime	0·015 – 0·25	0·06	0·015 – 0·5	0·23	0·015 – 1	0·15	0·015 – 0·5	0·33
Cefamandole	0·125 – 4	1·65	0·25 – 8	7·1	0·125 – 4	1·55	0·125 – 8	3·33
Cefoperazone	0·0018 – 0·25	0·11	0·0075 – 1	0·37	0·0037 – 0·125	0·10	0·0037 – 0·5	0·22
Moxalactam	0·015 – 0·125	0·06	0·015 – 1	0·12	0·015 – 0·125	0·06	0·015 – 1	0·21
Cefotaxime	0·0018 – 0·06	0·015	0·0018 – 0·25	0·13	0·0018 – 0·06	0·026	0·0018 – 0·25	0·197
Ceftriaxone	0·0018 – 0·015	0·004	0·0009 – 0·25	0·05	0·0018 – 0·015	0·007	0·0018 – 0·125	0·078

the Far East were generally more resistant to a panel of antibiotics than those isolated in the United States. In accordance with these earlier observations, we have found that gonococci isolated from six different south east Asian localities showed a decreased susceptibility to tetracycline, erythromycin, and kanamycin. Of the 88 strains tested, 49% required for inhibition concentrations of tetracycline ⩾2 μg/ml, 80% required concentrations of erythromycin ⩾2 μg/ml, and 34% required concentrations of kanamycin ⩾32 μg/ml respectively. Using the same MIC cut-off values this proportion of resistant strains was much greater than those reported in Europe and African countries[13] as well as those in the United States.[12] These MIC values were chosen since they are close to serum concentrations of these antibiotics attainable after drug administration. At least for tetracycline, an increase in treatment failure rate was shown to occur when the inhibitory concentrations of tetracycline for gonococcal strains reached 1·0 μg/ml.[14] High resistance to tetracycline, erythromycin, and kanamycin does not appear to be restricted to gonococci isolated from a particular south east Asian locality nor is it related to penicillinase production. Furthermore, concurrent resistance to two or three drugs is common among PPNG and non-PPNG isolates.

All strains examined were susceptible to sulphamethoxazole-trimethoprim (19:1 ratio) and spectinomycin. None of the isolates required for inhibition a concentration of these drugs >32 μg/ml. Co-trimoxazole has been used frequently for gonorrhoea treatment in south east Asia but, as has been reported recently in a limited clinical trial in Hong Kong,[15] all three cases treated with oral co-trimoxazole failed to respond. In contrast, five cases treated with a single dose of spectinomycin were cured. On the other hand, spectinomycin-resistant

strains of *N gonorrhoeae* have been isolated in Europe and the United States[16]; it is therefore important to monitor the sensitivity of PPNG isolates to this drug also.

Other than spectinomycin, all eight cephalosporins tested were effective against both PPNG and non-PPNG strains with the newer compounds showing the highest in vitro activities. In contrast to cefoxitin, cefuroxime, moxalactam, and cefoperazone, however, when the inoculum size was increased from 10^3 to 10^6 cfu there was a greater than fourfold increase in inhibitory concentrations for cefamandole, cefotaxime, and ceftriaxone. For the latter two compounds, while there was pronounced effect of inoculum size, the MIC_{90} against these isolates remained lower than 0·2 μg/ml. The effect of inoculum size for cefamandole was greatest with PPNG strains. This is in agreement with results reported by Phillips.[17] Syriopouls *et al*[18] have also shown an inoculum size effect for cefamandole in ampicillin-resistant *Haemophilus influenzae* strains, the R-factor mediated β-lactamase enzyme produced by *H influenza* and *N gonorrhoeae* being considered to be similar if not identical.[19]

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